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(54) **Title:** PREPARATION OF CELLULOSIC MATERIALS

(57) **Abstract:** The present invention provides methods and compositions for desizing, scouring and bleaching of cellulosic materials, which are carried out by contacting the cellulosic materials simultaneously or sequentially in a single-bath process with an enzyme system and a bleaching system comprising hydrogen peroxide or at least one peroxy compound which generates hydrogen peroxide when dissolved in water, or combinations thereof, and at least one bleach activator.

PREPARATION OF CELLULOSIC MATERIALS

Field of the Invention

The present invention relates to methods and compositions for treating cellulosic materials, and more particularly, to methods and compositions for desizing, scouring and bleaching cellulosic materials.

Background of the Invention

The processing of cellulosic material, such as, cotton fiber, into a material ready for garment manufacture involves several steps: spinning of the fiber into a yarn; construction of woven or knit fabric from the yarn; and subsequent preparation, dyeing and finishing operations. The preparation process, which may involve desizing (for woven goods), scouring, and bleaching, produces a textile suitable for dyeing or finishing.

A. Desizing: Woven goods are the prevalent form of textile fabric construction. The weaving process requires a "sizing" of the warp yarn to protect it from abrasion. The size must be removed after the weaving process as the first step in preparing the woven goods . Starch, polyvinyl alcohol, carboxymethyl cellulose, waxes and acrylic binders are examples of typical sizing agents commonly used in the industry. In order to ensure a high whiteness and/or a good dyeability, the size and other applied must be thoroughly removed. It is generally believed that an efficient desizing is crucial to the subsequent preparation processes, namely, the scouring and bleaching processes. The sized fabric, in either rope or open width form, is contacted with the processing liquid containing the desizing agent. The desizing agent employed depends upon the type of size to be removed. The most common sizing agent for cotton fabric is based upon starch. Therefore, most often, woven cotton fabrics are desized by a combination of hot water, the enzyme alpha amylase and a wetting agent or surfactant.

B. Scouring: The scouring process removes much of the non-cellulosic compounds naturally found in cotton. In addition to the natural non-cellulosic impurities, scouring can remove residual manufacturing introduced materials such as spinning, coning or slashing lubricants. Conventional scouring processes typically utilize highly alkaline chemical treatment, which results not only in removal of impurities but also in weakening of the underlying cellulose component of the fiber or fabric. The chemical scouring is followed by extensive rinsing to reduce the risk of re-depositing impurities. Insufficient rinsing yields alkaline residue and uneven removal of impurities

on the fabric, which in turn results in uneven dyeing in the subsequent process. Furthermore, chemical scouring creates environmental problems in effluent disposal, due to the chemicals employed and the materials extracted from the fibers. The use of enzymes for scouring has also been proposed as an alternative to chemical scouring processes, as described, e.g., in WO9824965, WO0071808, JP6220772, JP10088472, U.S. Patent No. 5,912,407; Hartzell et al., *Textile Res.* 68:233 (1998); Hsieh et al., *Textile Res.* 69:590 (1999); Buchert et al., *Text. Chem. Col. & Am. Dyestuff Reptr.* 32:48 (2000); and Li et al., *Text. Chem. Color.* 29:71 (1997).

C. Bleaching: Bleaching of textiles is the final preparation step in the manufacturing of textile fabrics and garments. The purpose of bleaching is to completely remove colored impurities, improve absorbency, and achieve adequate whiteness and dyeability. The most widely used bleaching process in the textile industry is the alkaline hydrogen peroxide process. A conventional textile bleach bath typically contains: sodium hydroxide, surfactant, optical brightener, stabilizers, and bleaching agents. The bleaching stage can be carried out in batch wise, semi-continuous, or continuous processes. When enzymes are used in either the desizing or scouring process, in order to obtain consistent, high quality results with commercial quantities of textiles, the desizing and scouring steps are usually performed separately from the bleaching step because it is very difficult to combine the enzymatic processes with alkaline peroxide bleaching in a single stage due to the high temperature and alkalinity requirement of alkaline peroxide bleaching.

Summary of the Invention

The present invention provides methods for single-bath desizing, scouring and bleaching of cellulosic materials, such as, crude fibers, yarn, or woven or knit textiles, made of cotton, linen, flax, ramie, rayon, hemp, jute, or blends of these fibers with each other or with other natural or synthetic fibers.

The methods are carried out by contacting the cellulosic materials with (i) an enzyme system, and (ii) a bleaching system; by adding the enzyme system and the bleaching system in the same solution containing the cellulosic material to be treated without emptying the bath or rinsing the cellulosic materials between desizing, scouring and bleaching steps, i.e., in a single-bath process. The enzyme system and the bleaching system may be added simultaneously to the solution. Alternatively, the enzyme system and the bleaching system may be added sequentially to the solution, in which the cellulosic materials are (i) contacted with the enzyme system for a sufficient time and under appropriate conditions that result in

effective bioscouring and/or desizing, after which (ii) the bleaching system is added directly to the solution containing cellulosic materials and the enzyme system.

In one embodiment of the present invention, a method for treating cellulosic material is disclosed, comprising contacting the cellulosic material with (i) an enzyme system for desizing and/or bioscouring the cellulosic material and (ii) a bleaching system comprising hydrogen peroxide or at least one compound which generates hydrogen peroxide when dissolved in water, or combinations thereof, and at least one bleach activator, wherein the enzyme system and the bleaching system are added simultaneously or sequentially to a single solution containing the cellulosic material.

In another embodiment, a method for treating cellulosic material is disclosed, comprising contacting the cellulosic material with (i) an enzyme system for desizing and/or bioscouring the cellulosic material and (ii) a bleaching system comprising hydrogen peroxide or at least one compound which generates hydrogen peroxide when dissolved in water, or combinations thereof, and at least one bleach activator, wherein the enzyme system and the bleaching system are added simultaneously or sequentially to a single solution containing the cellulosic material, and wherein the contacting is performed without the addition of alkali.

In yet another embodiment, a method for treating cellulosic material is disclosed, comprising contacting the cellulosic material with (i) an enzyme system for desizing and/or bioscouring the cellulosic material and (ii) a bleaching system comprising hydrogen peroxide or at least one compound which generates hydrogen peroxide when dissolved in water, or combinations thereof, and at least one bleach activator, wherein the enzyme system and the bleaching system are added simultaneously or sequentially to a single solution containing the cellulosic material, and wherein the contacting is performed at a high pH, preferably above 9.

The methods and compositions of present invention provide a product exhibiting a high wettability, high whiteness, and uniformity of mote removal, while having advantages over conventional preparation processes, including: (i) shorter processing times; (ii) conservation of water; and (iii) reduction in waste stream.

Detailed Description of the Invention

Cellulosic Materials

As used herein, a "cellulosic material" refers to the cellulosic substrate to be treated and comprises, without limitation, cotton, linen, flax, ramie, rayon, hemp, jute, and their blends with other natural or synthetic fibers. The cellulosic material may also comprise, without limitation, crude fiber, yarn, woven or knit textile or fabric, or a garment or finished product.

Enzyme System

As used in the present invention, an enzyme system refers to a bioscouring enzyme system and/or a desizing enzyme system. Accordingly, an enzyme system may comprise one or more bioscouring enzymes with or without one or more desizing enzymes or one or more desizing enzymes with or without one or more bioscouring enzymes. Preferably, the enzyme system is compatible with (i) the conditions in which bleaching is performed simultaneously with bioscouring and/or desizing processes or (ii) the conditions in which bleaching is performed sequentially with bioscouring and/or desizing processes, as described herein.

Desizing Enzymes:

Any suitable desizing enzyme may be used in the present invention. Preferably, the desizing enzyme is an amylolytic enzyme. More preferably, the desizing enzyme is an alpha or beta amylase and combinations thereof.

Alpha and beta amylases which are appropriate in the context of the present invention include those of bacterial or fungal origin. Chemically or genetically modified mutants of such amylases are also included in this connection. Preferred alpha-amylases include, for example, alpha-amylases obtainable from Bacillus species, in particular a special strain of *B. licheniformis*, described in more detail in GB 1296839. More preferred amylases include Duramyl™, Termamyl™, Fungamyl™ and BAN™ (all available from Novozymes A/S, Bagsvaerd, Denmark), and Rapidase™ and Maxamyl™ (available from Gist-Brocades, Holland). Other preferred amylolytic enzymes are CGTases (cyclodextrin glucanotransferases, EC 2.4.1.19), e.g., those obtained from species of Bacillus, Thermoanaerobactor or Thermoanaero-bacterium.

The desizing enzymes may also preferably be derived from the enzymes listed above in which one or more amino acids have been added, deleted, or substituted, including hybrid polypeptides, so long as the resulting polypeptides exhibit desizing activity. Such variants useful in practicing the present invention can be created using conventional mutagenesis procedures and identified using, e.g., high-throughput screening techniques such as the agar plate screening procedure.

The desizing enzyme is added to the aqueous solution or wash liquor (i.e., the treating composition) in an amount effective to desize the cellulosic materials. Typically, desizing enzymes, such as alpha-amylases, are incorporated into the treating composition in amount from 0.00001% to 2% of enzyme protein by weight of the composition, preferably in an amount from 0.0001% to 1% of enzyme protein by weight of the composition, more preferably in an amount from 0.001% to 0.5% of enzyme

protein by weight of the composition, and even more preferably in an amount from 0.01% to 0.2% of enzyme protein by weight of the composition. The desizing enzyme is preferably used at a level from about 2 to 30,000 KNU/l, more preferably 20-30,000 KNU/l and most preferably 200-300 KNU/l or from about 3-50,000 NAU/l, more preferably 30-5,000 NAU/l, most preferably 350-500 NAU/l.

Bioscouring Enzymes:

Any suitable bioscouring enzyme may be used in the present invention. Preferred bioscouring enzymes include, without limitation, pectinases, proteases, lipases, cutinases and combinations thereof, more preferably, the bioscouring enzyme is a pectinases, and even more preferably, the bioscouring enzyme is a pectate lyase.

Pectinases: Any pectinolytic enzyme composition with the ability to degrade the pectin composition of plant cell walls may be used in practicing the present invention. Suitable pectinases include, without limitation, those of fungal or bacterial origin. Chemically or genetically modified pectinases are also encompassed. Preferably, the pectinases used in the invention are recombinantly produced and are mono-component enzymes.

Pectinases can be classified according to their preferential substrate, highly methyl-esterified pectin or low methyl-esterified pectin and polygalacturonic acid (pectate), and their reaction mechanism, beta-elimination or hydrolysis. Pectinases can be mainly endo-acting, cutting the polymer at random sites within the chain to give a mixture of oligomers, or they may be exo-acting, attacking from one end of the polymer and producing monomers or dimers. Several pectinase activities acting on the smooth regions of pectin are included in the classification of enzymes provided by Enzyme Nomenclature (1992), e.g., pectate lyase (EC 4.2.2.2), pectin lyase (EC 4.2.2.10), polygalacturonase (EC 3.2.1.15), exo-polygalacturonase (EC 3.2.1.67), exo-polygalacturonate lyase (EC 4.2.2.9) and exo-poly-alpha-galacturonosidase (EC 3.2.1.82).

In preferred embodiments of the present invention, the pectinase is a pectate lyase. Pectate lyase enzymatic activity as used herein refers to catalysis of the random cleavage of α-1,4-glycosidic linkages in pectic acid (also called polygalcturonic acid) by transelimination. Pectate lyases are also termed polygalacturonate lyases and poly(1,4-α-D-galacturonide) lyases.

Any pectate lyase may be used in practicing the present invention. In preferred embodiments, the methods utilize a pectate lyase that exhibits maximal activity at temperatures above about 70°C. Pectate lyases may also preferably exhibit maximal activity at a pH above about 8 and/or exhibit enzymatic activity in the absence of added

divalent cations, such as, calcium ions. Non-limiting examples of pectate lyases for use in the present invention include pectate lyases that have been cloned from different bacterial genera such as *Erwinia*, *Pseudomonas*, *Klebsiella* and *Xanthomonas*, as well as from *Bacillus subtilis* (Nasser et al. (1993) *FEBS Letts.* 335:319-326) and *Bacillus* sp. YA-14 (Kim et al. (1994) *Biosci. Biotech. Biochem.* 58:947-949). Purification of pectate lyases with maximum activity in the pH range of 8-10 produced by *Bacillus pumilus* (Dave and Vaughn (1971) *J. Bacteriol.* 108:166-174), *B. polymyxa* (Nagel and Vaughn (1961) *Arch. Biochem. Biophys.* 93:344-352), *B. stearothermophilus* (Karbassi and Vaughn (1980) *Can. J. Microbiol.* 26:377-384), *Bacillus* sp. (Hasegawa and Nagel (1966) *J. Food Sci.* 31:838-845) and *Bacillus* sp. RK9 (Kelly and Fogarty (1978) *Can. J. Microbiol.* 24:1164-1172) have also been described. Any of the above, as well as divalent cation-independent and/or thermostable pectate lyases, may be used in practicing the invention. In preferred embodiments, the pectate lyase comprises the amino acid sequence of a pectate lyase disclosed in Heffron et al., (1995) *Mol. Plant-Microbe Interact.* 8: 331-334 and Henrissat et al., (1995) *Plant Physiol.* 107: 963-976.

The pectinases may be incorporated in the aqueous enzyme solution or wash liquor in an amount from 0.00001% to 2% of enzyme protein by weight of the composition, preferably in an amount from 0.0001% to 1% of enzyme protein by weight of the composition, more preferably in an amount from 0.001% to 0.5% of enzyme protein be weight to the composition, and even more preferably in an amount from 0.01% to 0.2% of enzyme protein by weight of the composition. Pectinases are preferably used at a level from about 2.5 to 500,000 APSU/g fabric, more preferably, at a level from about 25 to 50,000 APSU/g fabric, and most preferably at a level from about 250 to 5,000 APSU/g fabric.

Proteases: Any protease suitable for use in the present invention may be employed. Suitable proteases include those of animal, vegetable or microbial origin, preferably of microbial origin. Preferably, the protease may be a serine protease or a metalloprotease, more preferably, an alkaline microbial protease or a trypsin-like protease. Examples of proteases include aminopeptidases, including prolyl aminopeptidase (3.4.11.5), X-pro aminopeptidase (3.4.11.9), bacterial leucyl aminopeptidase (3.4.11.10), thermophilic aminopeptidase (3.4.11.12), lysyl aminopeptidase (3.4.11.15), tryptophanyl aminopeptidase (3.4.11.17), and methionyl aminopeptidase (3.4.11.18); serine endopeptidases, including chymotrypsin (3.4.21.1), trypsin (3.4.21.4), cucumisin (3.4.21.25), brachyurin (3.4.21.32), cerevisin (3.4.21.48) and subtilisin (3.4.21.62); cysteine endopeptidases, including papain (3.4.22.2), ficain (3.4.22.3), chymopapain (3.4.22.6), asclepain (3.4.22.7), actinidain (3.4.22.14), caricain (3.4.22.30) and ananain (3.4.22.31); aspartic endopeptidases, including pepsin A

(3.4.23.1), Aspergillopepsin I (3.4.23.18), Penicillopepsin (3.4.23.20) and Saccharopepsin (3.4.23.25); and metalloendopeptidases, including Bacillolysin (3.4.24.28).

Commercially available proteases include Alcalase, Savinase, Primase, Duralase, Esperase, Kannase, and Durazym (available from Novozymes A/S), Maxatase, Maxacal, Maxapem, Properase, Purafect, Purafect OxP, FN2, FN3 and FN4 (available from Genencor International Inc.).

Also useful in the present invention are protease variants, such as those disclosed in EP 130,756 (Genentech), EP 214,435 (Henkel), WO 87/04461 (Amgen), WO 87/05050 (Genex), EP 251.446 (Genencor), EP 260.105 (Genencor), Thomas et al., (1985), Nature. 318, p. 375-376, Thomas et al., (1987), J. Mol. Biol., 193, pp. 803-813, Russel et al., (1987), Nature, 328, p. 496-500, WO 88/08028 (Genex), WO 88/08033 (Amgen), WO 89/06279 (Novozymes A/S), WO 91/00345 (Novozymes A/S), EP 525 610 (Solvay) and WO 94/02618 (Gist-Brocades N.V.). The activity of proteases can be determined as described in "Methods of Enzymatic Analysis", third edition, 1984, Verlag Chemie, Weinheim, vol. 5.

Proteases are preferably incorporated into the aqueous enzyme solution or wash liquor in an amount from 0.00001% to 2% of enzyme protein by weight of the composition, preferably in an amount from 0.0001% to 1% of enzyme protein by weight of the composition, more preferably in an amount from 0.001% to 0.5% of enzyme protein be weight to the composition, and even more preferably in an amount from 0.01% to 0.2% of enzyme protein by weight of the composition.

Lipases: Any lipase suitable for use in the present invention may be used. Suitable lipases (also termed carboxylic ester hydrolases) preferably include those of bacterial or fungal origin, including triacylglycerol lipases (3.1.1.3) and Phospholipase A_2.(3.1.1.4.). Lipases for use in the present invention include, without limitation, lipases from *Humicola* (synonym *Thermomyces*), such as from *H. lanuginosa* (*T. lanuginosus*) as described in EP 258 068 and EP 305 216 or from *H. insolens* as described in WO 96/13580; a *Pseudomonas* lipase, such as from *P. alcaligenes* or *P. pseudoalcaligenes* (EP 218 272), *P. cepacia* (EP 331 376), *P. stutzeri* (GB 1,372,034), *P. fluorescens*, *Pseudomonas sp.* strain SD 705 (WO 95/06720 and WO 96/27002), *P. wisconsinensis* (WO 96/12012); a *Bacillus* lipase, such as from *B. subtilis* (Dartois et al., *Biochem.Biophys. Acta*, 1131:253-360, 1993); *B. stearothermophilus* (JP 64/744992) or *B. pumilus* (WO 91/16422). Other examples are lipase variants such as those described in WO 92/05249, WO 94/01541, EP 407 225, EP 260 105, WO 95/35381, WO 96/00292, WO 95/30744, WO 94/25578, WO 95/14783, WO 95/22615, WO 97/04079 and WO 97/07202. Preferred commercially available lipase enzymes include

Lipolase™ and Lipolase Ultra™, Lipozyme™ , Palatase™, Novozym™435, and Lecitase™ (all available from Novovozymes A/S). The activity of the lipase can be determined as described in "Methods of Enzymatic Analysis", Third Edition, 1984, Verlag Chemie, Weinhein, vol. 4.

Lipases are preferably incorporated in the aqueous enzyme solution or wash liquor in an amount from 0.00001% to 2% of enzyme protein by weight of the composition, preferably in an amount from 0.0001% to 1% of enzyme protein by weight of the composition, more preferably in an amount from 0.001% to 0.5% of enzyme protein be weight to the composition, and even more preferably in an amount from 0.01% to 0.2% of enzyme protein by weight of the composition.

Cutinases: Any cutinase suitable for use in the present invention may be used, including, for example, the cutinase derived from *Humicola insolens* cutinase strain DSM 1800, as described in Example 2 of U.S. Patent No. 4,810,414.

Cutinases are preferably incorporated in the aqueous enzyme solution in an amount from 0.00001% to 2% of enzyme protein by weight of the composition, preferably in an amount from 0.0001% to 1% of enzyme protein by weight of the composition, more preferably in an amount from 0.001% to 0.5% of enzyme protein be weight to the composition, and even more preferably in an amount from 0.01% to 0.2% of enzyme protein by weight of the composition.

Suitable bioscouring enzymes also include, for example, bioscouring enzymes derived from the enzymes listed above in which one or more amino acids have been added, deleted, or substituted, including hybrid polypeptides, may be used, so long as the resulting polypeptides exhibit bioscouring activity. Such variants useful in practicing the present invention can be created using conventional mutagenesis procedures and identified using, e.g., high-throughput screening techniques such as the agar plate screening procedure. For example, pectate lyase activity may be measured by applying a test solution to 4 mm holes punched out in agar plates (such as, for example, LB agar), containing 0.7% w/v sodium polygalacturonate (Sigma P 1879). The plates are then incubated for 6 h at a particular temperature (such as, e.g., 75°C). The plates are then soaked in either (i) 1M $CaCl_2$ for 0.5h or (ii) 1% mixed alkyl trimethylammonium Br (MTAB, Sigma M-7635) for 1 h. Both of these procedures cause the precipitation of polygalacturonate within the agar. Pectate lyase activity can be detected by the appearance of clear zones within a background of precipitated polygalacturonate. Sensitivity of the assay is calibrated using dilutions of a standard preparation of pectate lyase.

Effective scouring typically results in improvement in wettability, when measured using the drop test according to AATCC Test Method 39-1980. Preferably, the

wettability of the bleached fabric is 20 seconds or less, most preferably, 10 seconds or less.

Desizing and bioscouring enzymes for use in the invention may be derived from their cell of origin or may be recombinantly produced, and may be purified or isolated. As used herein, a "purified" or "isolated" enzyme is one that has been treated to remove non-enzyme material or other enzymes derived from the cell in which it was synthesized that could interfere with its enzymatic activity. Typically, the desizing and bioscouring enzyme is separated from the bacterial or fungal microorganism in which it is produced as an endogenous constituent or as a recombinant product. If the enzyme is secreted into the culture medium, purification may comprise separating the culture medium from the biomass by centrifugation, filtration, or precipitation, using conventional methods. Alternatively, the enzyme may be released from the host cell by cell disruption and separation of the biomass. In some cases, further purification may be achieved by conventional protein purification methods, including without limitation ammonium sulfate precipitation; acid or chaotrope extraction; ion-exchange, molecular sieve, and hydrophobic chromatography, including FPLC and HPLC; preparative isoelectric focusing; and preparative polyacrylamide gel electrophoresis. Alternatively, purification may be achieved using affinity chromatography, including immunoaffinity chromatography. For example, hybrid recombinant pectate lyases may be used having an additional amino acid sequence that serves as an affinity "tag", which facilitates purification using an appropriate solid-phase matrix.

The desizing and bioscouring enzyme used in the methods of the invention may also be chemically modified to enhance one or more properties that render them even more advantageous, such as, e.g., increasing solubility, decreasing lability or divalent ion dependence, etc. The modifications include, without limitation, phosphorylation, acetylation, sulfation, acylation, or other protein modifications known to those skilled in the art.

Bleaching Systems

Any bleaching system may be used in the present invention that is either compatible with (i) the conditions used for desizing and/or scouring, when desizing and/or scouring are performed simultaneously with the bleaching process, or (ii) the conditions used for desizing and/or scouring, when desizing and/or scouring are performed sequentially with the bleaching process. Preferably, the bleaching system comprises at least one bleach compound, at least one bleach activator, and, optionally, at least one bleach stabilizer, as described herein below.

Bleach Compound:

The bleach compound is preferably hydrogen peroxide or a compound which generate hydrogen peroxide when dissolved in water, such as, a peroxy compound. Examples of suitable compounds which generate hydrogen peroxide when dissolved in water are alkali metal perborates or alkali metal carbonate perhydrates, especially the sodium salts. The bleach compound hydrogen peroxide is preferably added to the aqueous solution or wash liquor in an amount from about .01 to about 10 g/l of the aqueous solution or wash liquor, more preferably in an amount from .1 to 5 g/l, most preferably, in an amount from .5 to 2.5 g/l. A compound which generates hydrogen peroxide, such as an alkali metal perborate or an alkali metal carbonate, is preferably added to the aqueous solution or wash liquor in an amount from about .001 to 20 g/l of the aqueous solution or wash liquor, more preferably, in an amount from about .1 to 10 g/l, and most preferably in an amount from about .5 to 5 g/l.

Bleach Activator:

Any suitable bleach activator may be employed in the present invention. The bleach activators preferred for use in accordance with the invention, include, for example, compounds of the following classes of substances: Polyacylated sugars or sugar derivatives with C sub 1-10 -acyl radicals, preferably acetyl, propionyl, octanoyl, nonanoyl or benzoyl radicals, particularly preferably acetyl radicals, can be used as bleach activators. Sugars or sugar derivatives which can be used are mono- or disaccharides and their reduced or oxidized derivatives, preferably glucose, mannose, fructose, sucrose, xylose or lactose. Particularly suitable bleach activators of this class of substances are, for example, pentaacetylglucose, xylose tetraacetate, 1-benzoyl-2,3,4,6-tetraacetylglucose and 1-octanoyl-2,3,4,6-tetraacetylglucose.

Another class of substances which are preferred for use as bleach activators in the present invention comprises acyloxybenzenesulfonic acids and their alkali metal and alkaline earth metal salts, such as C sub 1-14 -acyl radicals. Acetyl, propionyl,

octanoyl, nonanoyl and benzoyl radicals are preferred, especially acetyl radicals and nonanoyl radicals. Particularly suitable bleach activators in this class of substances are acetyloxybenzenesulfonic acid and benzoyloxybenzenesulfonic acid. They are preferably employed in the form of their sodium salts.

Other bleach activators for use in the present invention include MMA and OCL, alone or in combination with each other or with TAED; O-acyloxime esters, such as acetone O-acetyloxime, acetone O-benzoyloxime, bis(propylimino) carbonate, bis(cyclohexylimino) carbonate as a bleach activator. Acylated oximes which can be used as a bleach activator according to the invention are described, for example, in EP-A-0 028 432. Oxime esters which can be used as a bleach activator according to the invention are described, for example in EP-A-0 267 046.

Additional preferred bleach activators include N-acylcaprolactams, such as N-acetylcaprolactam, N-benzoylcaprolactam, N-octanoylcaprolactam and carbonylbiscaprolactam; N,N-diacylated and N,N,N',N'-tetraacylated amines, such as N,N,N',N'-tetraacetylmethylenediamine and -ethylenediamine (TAED), N,N-diacetylaniline, N,N-diacetyl-p-toluidine or 1,3-diacylated hydantoins such as 1,3-diacetyl-5,5-dimethylhydantoin; N-alkyl-N-sulfonylcarboxamides, such as N-methyl-N-mesylacetamide or N-methyl-N-mesylbenzamide; N-acylated cyclic hydrazides, acylated triazoles or urazoles, such as monoacetylated maleic hydrazide; O,N,N-trisubstituted hydroxylamines, such as O-benzoyl-N,N-succinyl-hydroxylamine, O-acetyl-N,N-succinylhydroxylamine or O,N,N-triacetylhydroxylamine; N,N'-diacylsulfamides, such as N,N'-dimethyl-N,N'-diacetylsulfamide or N,N'-diethyl-N,N'-dipropionylsulfamide; triacylcyanurates, such as triacetylcyanurate or tribenzoylcyanurate; carboxylic anhydrides, such as benzoic anhydride, m-chlorobenzoic anhydride or phthalic anhydride; 1,3-diacyl-4,5-diacyloxyimidazolines, such as 1,3-diacetyl-4,5-diacetoxyimidazoline; tetraacetylglycoluril and tetrapropionylglycoluril; diacylated 2,5-diketopiperazines, such as 1,4-diacetyl-2,5-diketopiperazine; acylation products of propylenediurea and 2,2-dimethylpropylenediurea, such as tetraacetylpropylenediurea; alpha.-acyloxypolyacylmalonamides, such as .alpha.-acetoxy-N,N'-diacetylmalonamide; diacyldioxohexahydro-1,3,5-triazines, such as 1,5-diacetyl-2,4-dioxohexahydro-1,3,5-triazine; 2-alkyl- or 2-aryl-(4H)-3,1-benzoxazin-4-ones as described, for example, in EP-B1-0 332 294 and EP-B 0 502 013, and 2-phenyl-(4H)-3,1-benzoxazin-4-one and 2-methyl-(4H)-3,1-benzoxazin-4-one, cationic nitrites, as described, for example, in EP 303 520 and EP 458 396 A1, such as, methosulfates or tosylates of trimethylammonioacetonitrile, N,N-dimethyl-N-octylammonioacetonitrile, 2-(trimethylammonio)propionitrile, 2-(trimethylammonio)-2-methylpropionitrile. Also

suitable are the methosulfates of N-methylpiperazinio-N,N'-diacetonitrile and N-methylmorpholinioacetonitrile (MMA).

Additional bleach activators for use in the present invention include percarbamic acids or diacyl percarbamates and precursors thereof.

Bleach activators are typically added in an amount from about .1 to 30 g/l, more preferably 0.5 to 10 g/l.

Bleach Stabilizer:

In another preferred embodiment of the present invention, the bleaching system additionally contains one or more bleach stabilizers. The bleach stabilizers comprise additives able to adsorb, bind or complex traces of heavy metals. Examples of additives which can be used according to the invention with a bleach-stabilizing action are polyanionic compounds, such as polyphosphates, polycarboxylates, polyhydroxypolycarboxylates, soluble silicates as completely or partially neutralized alkali metal or alkaline earth metal salts, in particular as neutral Na or Mg salts, which are relatively weak bleach stabilizers. Examples of strong bleach stabilizers which can be used according to the invention are complexing agents such as ethylenediaminetetraacetate (EDTA), diethylenetriaminepentaacetic acid (DTPA), nitrilotriacetic acid (NTA), methyl-glycinediacetic acid (MGDA), .beta.-alaninediacetic acid (ADA), ethylenediamine-N,N'-disuccinate (EDDS) and phosphonates such as ethylenediaminetetramethylenephosphonate, diethylenetriaminepentamethylenephosphonate (DTMPA) or hydroxyethylidene-1,1-diphosphonic acid in the form of the acids or as partially or completely neutralized alkali metal salts.

The bleach stabilizer is typically added to the treating composition in an amount from about .1 to about 5/g liter of the composition, more preferably from about .5 to about 2g/l, and most preferably about 1 g/l.

The bleach composition according to the invention preferably contains at least one bleach stabilizer, and more preferably, at least one of the above mentioned strong bleach stabilizers. Effective bleaching typically results in one or more of the following properties: (i) a desired whiteness (as determined by Ganz whiteness measurement using, e.g., a Macbeth color eye); (ii) a satisfactory uniformity of mote removal (assessed by visual examination); Preferably, the whiteness of the fabric is 50 Ganz units or higher, and most preferably, 60 Ganz units or higher.

Accordingly, in a preferred embodiment, the single-bath process comprises an enzyme system, such as, pectate lyase, hydrogen peroxide, a bleach activator (such as, TAED) and a bleach stabilizer.

Alkali Agents:

Alkali agents are well known in the art. Preferred alkali agents used in the present invention include, sodium hydroxide, sodium carbonate, sodium bicarbonate, sodium perborate, sodium sulfide and sodium sulfite. However, in some embodiments, it is preferred that the single-bath process is carried out in the absence of an alkali agent, in particular, when treating alkaline-sensitive cellulosic materials, such as, silk and wool.

Additional components:

In some embodiments of the invention, the aqueous solution or wash liquor further comprises other components, including without limitation other enzymes, as well as surfactants, antifoaming agents, lubricants, builder systems, and the like, that enhance the scouring and/or bleaching processes and/or provide superior effects related to, e.g., strength, resistance to pilling, water absorbency, and dyeability.

Enzymes suitable for use in the present invention include without limitation pectinases, proteases, and lipases as described above; and cellulases. Cellulases are classified in a series of enzyme families encompassing endo- and exo- activities as well as cellobiose hydrolyzing capability. The cellulase used in practicing the present invention may be derived from microorganisms which are known to be capable of producing cellulolytic enzymes, such as, e.g., species of *Humicola, Thermomyces, Bacillus, Trichoderma, Fusarium, Myceliophthora, Phanerochaete, Irpex, Scytalidium, Schizophyllum, Penicillium, Aspergillus*, or *Geotricum*, particularly *Humicola insolens, Fusarium oxysporum*, or *Trichoderma reesei*. Non-limiting examples of suitable cellulases are disclosed in U.S. Patent No. 4,435,307; European patent application No. 0 495 257; PCT Patent Application No. WO91/17244; and European Patent Application No. EP-A2-271 004.

The enzymes may be isolated from their cell of origin or may be recombinantly produced, and may be chemically or genetically modified. Typically, the enzymes are incorporated in the aqueous solution at a level of from about 0.0001% to about 1% of enzyme protein by weight of the composition, more preferably from about 0.001% to about 0.5% and most preferably from 0.01% to 0.2%. It will be understood that the amount of enzymatic activity units for each additional enzyme to be used in the methods of the present invention in conjunction with a particular bioscouring enzyme can be easily determined using conventional assays.

Surfactants suitable for use in practicing the present invention include, without limitation, nonionic (U.S. Patent No. 4,565,647); anionic; cationic; and zwitterionic surfactants (U.S. Patent No. 3,929,678); which are typically present at a concentration of between about 0.2% to about 15% by weight, preferably from about 1% to about

10% by weight. Anionic surfactants include, without limitation, linear alkylbenzenesulfonate, α-olefinsulfonate, alkyl sulfate (fatty alcohol sulfate), alcohol ethoxysulfate, secondary alkanesulfonate, alpha-sulfo fatty acid methyl ester, alkyl- or alkenylsuccinic acid, and soap. Non-ionic surfactants include, without limitation, alcohol ethoxylate, nonylphenol ethoxylate, alkylpolyglycoside, alkyldimethylamineoxide, ethoxylated fatty acid monoethanolamide, fatty acid monoethanolamide, polyhydroxy alkyl fatty acid amide, and N-acyl N-alkyl derivatives of glucosamine ("glucamides").

Builder systems include, without limitation, aluminosilicates, silicates, polycarboxylates and fatty acids, materials such as ethylenediamine tetraacetate, and metal ion sequestrants such as aminopolyphosphonates, particularly ethylenediamine tetramethylene phosphonic acid and diethylene triamine pentamethylenephosphonic acid, which are included at a concentration of between about 5% to 80% by weight, preferably between about 5% and about 30% by weight.

Antifoam agents include without limitation silicones (U.S. Patent No. 3,933,672; DC-544 (Dow Corning), which are typically included at a concentration of between about 0.01% and about 1% by weight.

The compositions may also contain soil-suspending agents, soil-releasing agents, optical brighteners, abrasives, and/or bactericides, as are conventionally known in the art.

Process conditions:

The manner in which the aqueous solution containing the enzyme and bleaching system is contacted with the cellulosic material will depend upon whether the processing regime is continuous, discontinuous pad-batch or batch. For example, for continuous or discontinuous pad-batch processing, the aqueous enzyme solution is preferably contained in a saturator bath and is applied continuously to the cellulosic material as it travels through the bath, during which process the cellulosic material typically absorbs the processing liquor at an amount of 0.5-1.5 times its weight. In batch operations, the cellulosic material is exposed to the enzyme solution for a period ranging from about 5 minutes to 24 hours at a liquor-to-fabric ratio of 5:1-50:1.

The aqueous solution or wash liquor typically has a pH of between about 4 and about 11. Preferably, the pH of the treating composition is between about 5 and about 10, preferably between about 7 to about 9, and most preferably about 8 to about 9.

In one embodiment, the single-bath method for treating cellulosic material is carried out without the addition of alkali. Preferably, this treatment is used for treating alkaline-sensitive cellulosic materials, such as silk and wool. In another embodiment,

the single-bath method for treating cellulosic material is carried out at a pH below 9, more preferably, below 8, and even more preferably below 7.

In another embodiment, the single-bath method for treating cellulosic material is carried out with the addition of alkaline. Preferably, the contacting is performed at a pH about 8 or above, more preferably at pH 9 or above, such as, at a pH from pH 9-11, preferably, 9.5-10.5, more preferably 10-11. The pH may be controlled, e.g., with the addition of an alkali agent, such as NaOH. Alkali agents may be added in amounts from about .1 to about 10% by wt. of fabric, as necessary to obtain the desired pH. However, as an artisan would appreciated, the amount of alkali agent added with depend on the amount of bleaching compound used.

The temperature at which the combined scouring and/or desizing and bleaching processes are carried out will depend on the process used. In the case of cold pad batch process, the scouring and/or desizing and bleaching temperature is preferably between about 15°C and about 45°C, and most preferably between about 25°C and about 35°C. For continuous and other batch processes, the scouring and/or desizing temperature is preferably between about 35°C and about 75°C, and most preferably between about 45°C and about 65°C; and the bleaching temperature may be between about 30°C and about 100°C, preferably between about 50°C and about 100°C, and most preferably between about 60°C and about 90°C.

It will be understood that the optimum dosage and concentration of the enzymes, bleaching compounds, bleach stabilizers, and alkali agents (if used), the volume of the aqueous solution or wash liquor, and the pH and temperature will vary, depending on: (i) the nature of the fiber, i.e., crude fiber, yarn, or textile; (ii) whether simultaneous or sequential scouring and bleaching are carried out; (iii) the particular enzyme(s) used, and the specific activity of the enzyme; (iv) the conditions of temperature, pH, time, etc., at which the processing occurs; (v) the presence of other components in the wash liquor; and (vi) the type of processing regime used, i.e., continuous, discontinuous pad-batch, or batch. The optimization of the process conditions can be determined using routine experimentation, such as, by establishing a matrix of conditions and testing different points in the matrix. For example, the amount of enzyme, the temperature at which the contacting occurs, and the total time of processing can be varied, after which the resulting cellulosic materials or textile is evaluated for (a) pectin removal; (b) a scoured property such as, e.g., wettability; and (c) quality of bleaching, such as whiteness.

In a preferred embodiment, the conditions or treating composition may be adjusted to favor the desizing, scouring or bleaching processes, such as, by adjusting pH, concentration of wetting agent, or concentration of divalent cationic chelator such

as ethylene diamine tetraacetate so as to further promote the bleaching process. In a preferred embodiment, the sequential mode may further comprise adjusting one or more properties of the composition of the aqueous solution or wash liquor between steps (ii) and (iii). For example, pH, concentration of wetting agent, or concentration of divalent cationic chelator, such as, ethylene diamine tetraacetate, may be adjusted between steps (ii) and (iii) so as to further promote the bleaching process. The conditions of the first and second incubations may also differ with respect to temperature, agitation, time, and the like.

The following are intended as non-limiting illustrations of the present invention.

Example 1: **Simultaneous Bioscouring and Bleaching with H_2O_2**

A. ***Bioscouring and Bleaching***: A 45 cm x 21.5 cm fabric weighing about 25 gram was cut from an interlock knit fabric (type 4600, Ramseur Co., NC). The fabric was loaded into a Labomat beaker (Mathis Labomat, Werner Mathis USA, Inc, NC), which was then filled with 250 mL of 20mM sodium phosphate buffer solution (pH9.2) containing 3000 APSU/kg fiber of pectate lyase, 0.5g/l wetting agent (Kierlon Jet B, BASF), 1.7g/L H_2O_2, and 0.75g/l stabilizer (Calgon, Dexter). The fabric was treated at 55°C for 15 minutes after which temperature was raised at 5°C /minute to 70°C for 1 hour. The fabric was then washed thoroughly with tap water to remove the residual chemicals and dried at room temperature overnight.

B. ***Analysis***: Whiteness of the fabric was measured by a Macbeth color eye in Ganz units. Wettability was determined by a drop test, measuring the time in seconds for a drop of water to be absorbed by the fabric.

The results are presented in Table 1. Both the whiteness and wettability of the fabric were very low. This example illustrates that bleaching the knitted fabric with hydrogen peroxide alone in the absence of alkali or bleach activators resulted in limited improvement in whiteness, wettability and mote removal.

Example 2: **Simultaneous Bioscouring and Bleaching with H_2O_2/TAED**

The same fabric and equipment were used as in Example 1 above. The experiment was conducted in essentially the same manner as example 1, except that 20mmol TAED (Aldrich) was added to the bioscouring/bleaching solution.

The results are shown in Table 1. The presence of TAED in the bioscouring/bleaching bath dramatically improved the whiteness and wettability of the fabric. It also resulted in significant mote removal.

Example 3: Simultaneous Bioscouring and Bleaching with H_2O_2/TAED/NaOH

The same fabric and equipment were used as in Example 1 above. The experiment was conducted in essentially the same manner as example 1, except that 2g/l NaOH was added to the bioscouring/bleaching solution.

The results are shown in Table 1. Surprisingly, unlike conventional peroxide bleaching, addition of sodium hydroxide in the bioscouring/bleaching bath did not further improve the whiteness and wettability of the fabric. In fact, it had some negative impact on the whiteness and mote removal efficiency.

Example 4: Sequential Bioscouring and Bleaching with H_2O_2/TAED

A. ***Bioscouring:*** A 45 cm x 21.5 cm fabric weighing about 25 gram was cut from an interlock knit fabric (type 4600, Ramseur Co., NC). The fabric was loaded into a Labomat beaker (Mathis Labomat, Werner Mathis USA, Inc, NC), which was then filled with 250 mL of 20mM sodium phosphate buffer solution (pH9.2) containing 3000 APSU/kg fiber of pectate lyase and 0.5g/l wetting agent (Kierlon Jet B, BASF). The fabric was treated at 55°C for 15 minutes.

B. ***Bleaching***: To the same beaker, add H_2O_2, TAED and Calgon. The final concentrations of H_2O_2, TAED, and Calgon were the same as Example 2 above. The Labomat temperature was raised at 5°C /minute to 70°C for 1 hour, after which the water was drained. The fabric was then washed thoroughly with tap water to remove the residual alkali and dried at room temperature.

The results are shown in Table 1. It is evident that the whiteness and wettability of the fabric from sequential bioscouring and bleaching process mode is better than that of the simultaneous mode (Example 2). The overall quality of the fabric was the best among the fabrics of Examples 1-10.

Example 5: Sequential Scouring and Bleaching with H_2O_2/TAED

The same fabric and equipment were used as described in Examples 1-4 above. The experiment was conducted in essentially the same manner as example 4 above, except that pectate lyase was absent from the scouring solution.

The results of whiteness and wettability are presented in Table 1 below. The wettability of the fabric is very poor. This example demonstrates that the presence of a bioscouring enzyme is critical to the wettability of the fabric.

Example 6: Sequential Bioscouring and Bleaching with H_2O_2/TAED/NaOH

The same fabric and equipment were used as described in Examples 1-4 above. The experiment was conducted in essentially the same manner as example 4 above, except that 2g/l NaOH was added to the bleaching solution.

The results were shown in Table 1 below. The whiteness of the fabric is much lower than that of Example 4. This example further illustrates that addition of sodium hydroxide to the bleach bath will negatively affect the whiteness of the fabric.

Table1. Single-bath bioscouring and bleaching of knitted fabrics

Example #	Process Mode	Scouring	Bleaching	Whiteness,Ganz 82	Wettability, Seconds	Motes
Starting Fabric				8.5	>60	5
1	Single-bath Simultaneous	Enzyme	Peroxide	44.6	33	4
2	Single-bath Simultaneous	Enzyme	Peroxide/ TAED	62.3	9	1
3	Single-bath Simultaneous	Enzyme	Peroxide/ TAED/NaOH	59.7	10	2
4	Single-bath Sequential	Enzyme	Peroxide/ TAED	63.4	2	1
5	Single-bath Sequential	Buffer	Peroxide/ TAED	63.0	23	1
6	Single-bath Sequential	Enzyme	Peroxide/ TAED/NaOH	59.8	2	1

*Rating of motes: 1: the fewest; 5: the most.

Example 7: **Two-bath Scouring and Bleaching**

The experiment was conducted in essentially the same manner as example 4 above, except that the scouring solution was drained and replaced with water after the bioscouring stage.

The results are shown in Table 2 below. The wettability of the fabric is good, but the whiteness and number of motes removed from the fabric are much lower than that from the simultaneous (Example 2) and sequential (Example 4) process mode.

Example 8: **Two-bath Bioscouring and Bleaching**

The experiment was conducted in essentially the same manner as example 7 above, except that pectate lyase was absent from the scouring solution.

The results are shown in Table 2. Because of the absence of pectate lyase from the bioscouring solution, the wettability of the fabric was very poor. This example further demonstrates that bioscouring enzyme is very important for improving the wettability of the fabric.

Example 9: Two-bath Scouring and Bleaching

The experiment was conducted in essentially the same manner as example 7 above, except that 2g/l NaOH was added to the bleaching liquor.

The results are shown in Table 2. Addition of sodium hydroxide to the bleaching bath improved the whiteness of the fabric. This is contrary to what has been observed in the simultaneous and sequential process mode.

Example 10: Two-bath Bioscouring and Bleaching

The experiment was conducted in essentially the same manner as example 9 above, except that pectate lyase was absent from the scouring solution.

The results are shown in Table 1. This example further illustrates addition of sodium hydroxide is necessary to improve the whiteness of the fabric in the two-stage process mode.

Table2. Two-bath bioscouring and bleaching of knitted fabrics

Example #	Process Mode	Scouring	Bleaching	Whiteness,Ganz 82	Wettability, Seconds	Motes
7	Two-bath	Enzyme	Peroxide/TAED	57.7	4	3
8	Two-bath	Buffer	Peroxide/TAED	56.9	>60	3
9	Two-bath	Enzyme	Peroxide/TAED/NaOH	59.1	5	2
10	Two-bath	Buffer	Peroxide/TAED/NaOH	59.6	10	2

*Rating of motes: 1: the fewest; 5: the most.

Example 11: Sequential Bioscouring and Bleaching with H_2O_2/NaOH/TAED

A. ***Bioscouring:*** Fabric swatches were cut from 100% cotton knit interlock (type 4600, Ramseur Co, NC). The size of swatch is 19 cm x 19.5 cm and each swatch is about

7.5 gram. Two swatches were loaded into a Labomat beaker (Mathis Labomat, Werner Mathis USA, Inc. NC), which was then filled with 150ml of 5mM sodium bicarbonate buffer (pH9.0) containing 3000 APSU/kg fabric of pectate lyase and 0.5 g/l wetting agent (Kierlon Jet B, BASF). The fabric was then treated at 55θC for 15 minutes.

B. ***Bleaching:*** To the same beaker, add 1g/l stabilizer (Prostogen N-S, BASF), 2g/l NaOH, 2.5g/l H_2O_2 and 1.32 g/l TAED. The Labomat temperature was raised at 3θC/minute to 70θC for 1 hour. After which the water was drained. The fabric was then washed thoroughly with tap water to remove the residual alkali and dried at room temperature.

The results are shown in Table 3. Due to the addition of NaOH, the solution pH was 10.83 at the end of the reaction. The whiteness of the fabric was measured by a Macbeth color eye in Ganz 82 unit. After enzymatic scouring and bleaching with peroxide/NaOH/TAED, the whiteness has reached 67.61. The fabric wettability is less than 1 second in a drop test according to AATCC Test Method 79-1995. Motes on the fabric almost completely disappeared.

Example 12: <u>Sequential Bioscouring and Bleaching with H_2O_2/NaOH</u>

The same fabric and equipment were used as described in Example 11. The experiment was conducted in essentially the same manner as Example 11 above, except that TAED was not added in the bleaching solution.

The results are shown in Table 3. The ending pH of reaction solution is 11.06, which is higher than that in Example 11. The whiteness is 62.64, which is much lower than the fabric whiteness in Example 11. A few motes are shown on fabric after the treatment. The higher ending pH, more motes, and lower fabric whiteness are due to the absence of TAED, thus a stronger bleaching agent is not generated in Example 12. Excellent wettability of fabric is also observed as indicated by 3 second wetting time, which indicates the effectiveness of enzymatic scouring.

Example 13: **Sequential Buffer Scouring and Bleaching with H_2O_2/NaOH/TAED**

The same fabric and equipment were used as described in Example 11. The experiment was conducted in essentially the same manner as Example 11 above, except that pectate lyase was not added in the bioscouring solution.

The results are shown in Table 3. The ending pH of reaction solution is 10.88, similar to that in Example 11. There are almost no motes observed on fabric after scouring and bleaching, also similar to that observed in Example 11. The fabric whiteness is 64.66, which is lower than fabric whiteness in Example 11. Fabric wettability is over 60 seconds, which is much worse than that in Example 11. The lower wettability and whiteness are due to the absence of enzyme in scouring.

Table 3

Example #	Scouring	Bleaching	Ending pH	Whiteness (Ganz 82)	Wettability (Seconds)	Motes*
Starting Fabric				8.5	>60	5
11	Enzyme	Peroxide/NaOH/TAED	10.83	67.61	<1	1
12	Enzyme	Peroxide/NaOH	11.06	62.64	3	2
13	Buffer	Peroxide/NaOH/TAED	10.88	64.66	>60	1

*Rating of Motes: 1: the fewest; 5: the most.

Example 14: **Sequential Bioscouring and Bleaching with H_2O_2/NaOH/TAED**

A. Bioscouring: A 19cm x 19.5 cm fabric swatch weighing about 7.5 gram was cut from 100% cotton knit interlock (type 4600, Ramseur Co, NC). Two swatches were loaded into a Labomat beaker (Mathis Labomat, Werner Mathis USA, Inc. NC), which was then filled with 150ml of 5mM sodium bicarbonate buffer (pH9.0) containing 0.1 g/l of pectate lyase (i.e. 3000APSU/kg fabric) and 0.5 g/l wetting agent (Kierlon Jet B, BASF). The fabric was then treated at 55θC for 15 minutes.

B. Bleaching: To the same beaker, add 4g/l stabilizer (Prostogen N-S, BASF), 4g/l NaOH, 10g/l H_2O_2 and 5.3 g/l TAED. The Labomat temperature was raised at 3θC/minute to 70θC for 1 hour. After which the water was drained. The fabric was then

washed thoroughly with tap water to remove the residual alkali, and it was dried at room temperature.

The results are shown in Table 4. Compared to Example 11, the same enzymatic scouring is performed in this example. Less than 1 second water absorbency time indicates that excellent fabric wettability is obtained. On the other hand, the bleaching is conducted at higher chemical dose including higher concentrations of NaOH, H_2O_2, stabilizer and TAED. As the consequence of higher chemical concentration, a higher fabric whiteness 72.46 (Ganz 82) was obtained compared to fabric whiteness 67.61 obtained in Example 11. All motes on fabric were completely removed in this example.

Example 15: **Sequential Bioscouring and Bleaching with NaOH/H_2O_2**

The same fabric and equipment were used as described in Example 14. The experiment was conducted in essentially the same manner as in Example 14 above, except that 2g/l stabilizer, 5g/l H2O2 were used in the bleaching solution.

As indicated in Table 4, fabric has whiteness of 71.56. The fabric whiteness is lower than that in Example 14, which indicates the effectiveness of TAED. Fabric also has an excellent wettability as indicated by less than 1 second of water absorbency in drop test. Motes were completely removed.

Example 16: **Sequential Buffer Scouring and Bleaching with H_2O_2/NaOH/TAED**

The same fabric and equipment were used as described in Example 14. The experiment was conducted in essentially the same manner as Example 14 above, except that pectate lyase was not added in the bioscouring solution.

The results are shown in Table 4. Compared to the results obtained in Example 14, fabric in this example does not have industrial acceptable wettability (e.g. <5 second). Due to lack of scouring effect, fabric whiteness is also lower than that in Example 14. Motes on fabric were completely removed.

Example 17: Sequential Buffer Scouring and Bleaching with $NaOH/H_2O_2$

The same fabric and equipment were used as described in Example 14. The experiment was conducted in essentially the same manner as Example 14 above, except that pectate lyase was not added in the bioscouring solution and TAED was not added in the bleaching solution.

The results are shown in Table 4. Much longer water absorbency time than that in Example 15 has demonstrated the effectiveness of pectate lyase enzyme in scouring. However, shorter water absorbency time than that in example 6 indicates that addition of TAED has no or negative impact on fabric wettability. On the other hand, fabric whiteness 71.17 is lower that fabric whiteness 71.94 in Example 16. This indicates again that the addition of TAED results in an increase of fabric whiteness.

Table 4

Example #	Scouring	Bleaching	CIE Whiteness, (Ganz 82)	Wettability (Seconds)	Motes
14	Enzyme	Peroxide/NaOH/TAED	72.46	<1	None
15	Enzyme	Peroxide/NaOH	71.56	<1	None
16	Buffer	Peroxide/NaOH/TAED	71.94	>60	None
17	Buffer	Peroxide/NaOH	71.17	46	None

Example 18: Effect of NaOH in Sequential Bioscouring and Bleaching

A. Bioscouring: A 19cm x 19.5 cm fabric swatch weighing about 7.5 gram was cut from 100% cotton knit interlock (type 4600, Ramseur Co, NC). Two swatches were loaded into a Labomat beaker (Mathis Labomat, Werner Mathis USA, Inc. NC), which was then filled with 150ml of 5mM sodium bicarbonate buffer (pH9.0) containing 0.1 g/l of pectate lyase (i.e.3000APSU/kg fabric) and 0.5 g/l wetting agent (Kierlon Jet B, BASF). The fabric was then treated at 55ØC for 15 minutes.

B. Bleaching: To the same beaker, add 1g/l stabilizer (Prostogen N-S, BASF), 2.5g/l H_2O_2 (Dexter Chemical) and 1.32 g/l TAED (Peractive AN, which has 84-88% active TAED content, from Clariant Co.). Therefore, the molar ratio of H_2O_2 to TAED is 14.7. NaOH concentration varies from 0-4 g/l. The Labomat temperature was raised at

3θC/minute to 70θC for 1 hour. After measuring the liquid pH at the end, the water was drained. The fabric was then washed thoroughly with tap water to remove the residual alkali, and it was dried at room temperature.

The results are shown in Table 5. As NaOH concentration increases, the ending pH increases, the wettability measured as water absorbency time in seconds increases as shown in Table 5 The fabric whiteness in Ganz 82. Fabric whiteness does not increase initially with small amount addition of NaOH (corresponding to ending pH range 5-8). Fabric whiteness increases substantially when relatively large amount of NaOH is added to the bleaching bath.

Example 19: **Effect of NaOH in Sequential Bioscouring and Bleaching**

The same fabric and equipment were used as described in Example 18. The experiment was conducted in essentially the same manner as Example 18 above, except that 2.65 g/l TAED was used instead of 1.32 g/l. Therefore, the molar ratio of H_2O_2 to TAED is 7.35 in this example.

The results are shown in Table 5. Very similar results and conclusions are obtained. As NaOH concentration increases, the ending pH increases, the wettability measured as water absorbency time in seconds increases as shown in Table 5. Fabric whiteness does not increase initially with small amount addition of NaOH (corresponding to ending pH range 5-8). Fabric whiteness increases substantially when relatively large amount of NaOH is added to the bleaching bath.

Furthermore, by comparing fabric whiteness of sample G in this example and sample A in Example 18, it is evident that higher TAED dose in fabric G treatment gives higher whiteness when no NaOH is added. This indicates the effectiveness of TAED at acidic conditions. However, when large amount of NaOH is added (e.g. fabric e vs. j), no substantial whiteness difference is observed by the increase TAED from 1.32g/l to 2.65g/l.

Table 5:

Example	Sample	NaOH(g/l)	H2O2/TAED Molar Ratio	Final pH	CIE Whiteness	Wetting
18	A	0	15	5.42	50.21	13
	B	0.5	15	7.78	52.08	16
	C	1	15	9.88	59.32	2
	D	2	15	10.92	64.59	<1
	E	3	15	11.41	65.98	<1
	F	4	15	11.74	67.14	<1
19	G	0	7	4.85	54.89	2
	H	1	7	8.48	55.29	6
	I	2	7	10.64	66.02	2
	J	3	7	11.18	66.76	<1
	K	4	7	11.73	66.56	2

All patents, patent applications, and literature references referred to herein are hereby incorporated by reference in their entirety. Many variations of the present invention will suggest themselves to those skilled in the art in light of the above detailed description. Such obvious variations are within the full-intended scope of the appended claims.

Claims:

1. A method for treating cellulosic material, comprising contacting the cellulosic material with (i) an enzyme system for desizing and/or bioscouring the cellulosic material and (ii) a bleaching system comprising hydrogen peroxide or at least one compound which generates hydrogen peroxide when dissolved in water, or combinations thereof, and at least one bleach activator, wherein the enzyme system and the bleaching system are added simultaneously or sequentially to a single solution containing the cellulosic material.

2. The method of claim 1, wherein the contacting is performed without the addition of alkali.

3. The method of claim 1 or 2, wherein the enzyme system and the bleaching system are added simultaneously to the solution containing the cellulosic material.

4. The method of claim 1 or 2, wherein the enzyme system and the bleaching system are added sequentially to the solution containing the cellulosic material, comprising (i) adding the enzyme system and incubating, and subsequently (ii) adding the bleaching system and incubating.

5. The method of claim 1 or 2, wherein the cellulosic material is contacted with the enzyme system and the bleaching system to produce a fabric with wettability of 20 seconds or less and whiteness of at least 50 Ganz units.

6. The method of claim 1 or 2, wherein the cellulosic material is (i) contacted with the enzyme system to produce a fabric with wettability of 20 seconds or less, after which (ii) the bleaching system is added to the solution containing the cellulosic material.

7. The method of claims 4, further comprising between said (i) and said (ii), adjusting a property of the solution selected from the group consisting of pH, ionic strength, temperature, concentration of surfactant, concentration of divalent cationic chelator, and combinations of any of the foregoing.

8. The method of claim 1 or 2, wherein the enzyme system comprises at least one enzyme for desizing the cellulosic material and at least one enzyme for bioscouring the cellulosic material

9. The method of claim 1 or 2, wherein the enzyme system is a bioscouring enzyme system.

10. The method of claim 1 or 2, wherein the enzyme system is a desizing enzyme system.

11. The method of claim 1 or 2, wherein the desizing enzyme system comprises at least one desizing enzyme selected from the group consisting of an alpha-amylase and a beta-amylase, and combinations thereof.

12. The method of claim 1 or 2, wherein the bioscouring enzyme system comprises at least one bioscouring enzyme selected from the group consisting of pectinase, protease, lipase, and combinations of any of the foregoing.

13. The method of claim 1 or 2, wherein the bioscouring enzyme system comprises at least one bioscouring enzyme selected from the group consisting of pectate lyase, pectin lyase, polygalacturonase, exo-polygalacturonase, exo-polygalacturonate lyase and exo-poly-alpha-galacturonosidase.

14. The method of claim 1 or 2, wherein the bioscouring enzyme system comprises a pectate lyase.

15. The method of claim 1 or 2, wherein the bioscouring enzyme system comprises a protease selected from the group consisting of aminopeptidases, serine endopeptidases, cysteine endopeptidases, aspartyl endopeptidases, and metalloendopeptidases.

16. The method of claim 1 or 2, wherein the bioscouring enzyme system comprises a lipase selected from the group consisting of triacylglycerol lipases and phospholipases.

17. The method of claim 1 or 2, wherein the bioscouring enzyme system comprises a pectate lyase that exhibits maximal pectate lyase enzymatic activity at a temperature above about 70°C.

18. The method of claim 1 or 2, wherein the bioscouring enzyme system comprises a pectate lyase that exhibits maximal pectate lyase enzymatic activity at a pH above about 8.

19. The method of claim 1 or 2, wherein the bleach activator is selected from the following class of substances: N-acylcaprolactams, N,N-diacylated and N,N,N',N'-tetraacylated amines,
O-acyloxime esters, N-alkyl-N-sulfonylcarboxamides, N-acylated cyclic hydrazides, O,N,N-trisubstituted hydroxylamines, N,N'-diacylsulfamides, triacylcyanurates, carboxylic anhydrides, acyloxybenzenesulfonic acids and their alkali metal and alkaline earth metal salts, 1,3-diacyl-4,5-diacyloxyimidazolines, diacylated 2,5-diketopiperazines, acylation products of propylenediurea and 2,2-dimethylpropylenediurea, alpha.-acyloxypolyacylmalonamides, diacyldioxohexahydro-1,3,5-triazines, 2-alkyl- or 2-aryl-(4H)-3,1-benzoxazin-4-ones, cationic nitrites and polyacylated sugars or sugar derivatives with C.sub.1-10 -acyl radicals.

20. The method of claim 19, wherein the bleaching system comprises a bleach stabilizer selected from the group consisting of ethylenediaminetetraacetate (EDTA), diethylenetriaminepentaacetic acid (DTPA), nitrilotriacetic acid (NTA), methyl-glycinediacetic acid (MGDA), .beta.-alaninediacetic acid (ADA), ethylenediamine-N,N'-disuccinate (EDDS), ethylenediaminetetramethylenephosphonate, diethylenetriaminepentamethylenephosphonate (DTMPA) or hydroxyethylidene-1,1-diphosphonic acid.

21. The method of claim 1 or 2, wherein the cellulosic materials comprise a textile.

22. The method of claim 22, wherein said textile is cotton.

23. The method of claim 1 or 2, wherein said single-bath solution further comprises one or more buffers, surfactants, chelating agents, and/or lubricants, or salts of any of the foregoing.

24. A method for treating cellulosic material, comprising contacting the cellulosic material with (i) an enzyme system for desizing and/or bioscouring the cellulosic material and (ii) a bleaching system comprising hydrogen peroxide or at least one compound which generates hydrogen peroxide when dissolved in water, or combinations thereof, and at least one bleach activator, wherein the enzyme system and the bleaching system are added simultaneously or sequentially to a single solution containing the cellulosic material, and wherein the contacting is carried out at a pH above 8.

25. The method of claim 24, wherein the contacting is carried out at a pH of 9 or above.

26. The method of claim 24, wherein the contacting is carried out at a pH from 9 to 11.

27. The method of claim 24, wherein the contacting is carried out at a pH from 10 to 11.

26. The method of claim 24, wherein the enzyme system and the bleaching system are added simultaneously to the solution containing the cellulosic material.

27. The method of claim 24, wherein the enzyme system and the bleaching system are added sequentially to the solution containing the cellulosic material, comprising (i) adding the enzyme system and incubating, and subsequently (ii) adding the bleaching system and incubating.

28. The method of claims 27, further comprising between said (i) and said (ii), adjusting a property of the solution selected from the group consisting of pH, ionic strength, temperature, concentration of surfactant, concentration of divalent cationic chelator, and combinations of any of the foregoing.

29. The method of claim 24, wherein the enzyme system comprises at least one enzyme for desizing the cellulosic material and at least one enzyme for bioscouring the cellulosic material

30. The method of claim 24, wherein the enzyme system is a bioscouring enzyme system.

31. The method of claim 24, wherein the enzyme system is a desizing enzyme system.

32. The method of claim 30, wherein the bioscouring enzyme system comprises a pectate lyase.

33. The method of claim 30, wherein the bioscouring enzyme system comprises a pectate lyase that exhibits maximal pectate lyase enzymatic activity at a pH above about 8.

34. The method of claim 24, wherein the at least one bleach activator is selected from the following class of substances:
: N-acylcaprolactams, N,N-diacylated and N,N,N',N'-tetraacylated amines, O-acyloxime esters, N-alkyl-N-sulfonylcarboxamides, N-acylated cyclic hydrazides, O,N,N-trisubstituted hydroxylamines, N,N'-diacylsulfamides, triacylcyanurates, carboxylic anhydrides, acyloxybenzenesulfonic acids and their alkali metal and alkaline earth metal salts, 1,3-diacyl-4,5-diacyloxyimidazolines, diacylated 2,5-diketopiperazines, acylation products of propylenediurea and 2,2-dimethylpropylenediurea, alpha.-acyloxypolyacylmalonamides, diacyldioxohexahydro-1,3,5-triazines, 2-alkyl- or 2-aryl-(4H)-3,1-benzoxazin-4-ones, cationic nitrites and polyacylated sugars or sugar derivatives with C.sub.1-10 -acyl radicals.

35. The method of claim 24 wherein the bleaching system comprises a bleach stabilizer selected from the group consisting of ethylenediaminetetraacetate (EDTA), diethylenetriaminepentaacetic acid (DTPA), nitrilotriacetic acid (NTA), methyl-glycinediacetic acid (MGDA), .beta.-alaninediacetic acid (ADA), ethylenediamine-N,N'-disuccinate (EDDS), ethylenediaminetetramethylenephosphonate, diethylenetriaminepentamethylenephosphonate (DTMPA) or hydroxyethylidene-1,1-diphosphonic acid.

36. The method of claim 24, wherein the cellulosic materials comprises a textile.

37. A method for treating cellulosic material, comprising contacting the cellulosic material with (i) an enzyme system for desizing and/or bioscouring the cellulosic material and (ii) a bleaching system comprising hydrogen peroxide or at least one

compound which generates hydrogen peroxide when dissolved in water, or combinations thereof, and at least one bleach activator, wherein the enzyme system and the bleaching system are added simultaneously or sequentially to a single solution containing the cellulosic material, and wherein the contacting is carried out without the addition of alkaline.

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A. CLASSIFICATION OF SUBJECT MATTER

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US CL : 510/276, 320, 392, 375

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 510/276, 320, 392, 375

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

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C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	US 6,380,146 A (BREEL et al.) 30 April 2002 (30.04.2002), entire document.	1-37

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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